testosterone in a biological fluid, wherein said free portion of testosterone is in equilibrium with another portion of the testosterone bound to one or more endogenous binders in said fluid comprising the steps of (a) forming a mixture of a sample of said fluid with (1) an amount of a specific antibody for the free portion of testosterone insufficient to substantially affect said equilibrium, and (2) a labeled analog of testosterone which is radioiodinated 6-hydroxy-testosterone-19-carboxymethyl ether histamine that binds to said antibody and has affinity for the engodenous binders lower than that of the free portion of testosterone for said endogenous binders, (b) maintaining said mixture to permit said labeled analog to compete with the free portion of testosterone for binding with the antibody, (c) measuring the amount of said labeled analog that has, or has not, become bound to the antibody, and (d) determining the concentration of the free portion of testosterone from said measurement, wherein the improvement comprises including in the mixture an amount of a blocking agent which is sulfobromophthalein to inhibit the binding of said labeled analog to the endogenous binders without displacing testosterone bound to said endogenous binders.

REMARKS

As noted the editorial corrections to the Specifications will take place upon allowance.

Turning to paragraph 8 of the Office Action, the support for the claim appears in the Specification, particularly pages 25-27.

To more closely track the Specification, we have amended the claim to recite "analog" rather than "derivative", however, they are the same thing.

The invention of this claims resides in the use of sulfobromophthalein as a blocking agent to reduce the binding of the labeled analog ("analog tracer") or derivative, which is specified in the claim to be radioiodinated 6-hydroxy-testosterone-19-carboxymethyl ether histamine, to the endogenous binder (primarily, albumin) naturally present in the sample. The Examiner will appreciate that the cited passage discloses a free testosterone measurement or assay in a biological fluid which relies on competitive binding between free testosterone and the specified radiolabeled testosterone analog to an antibody. There exists a natural equilibrium in the patient sample between free testosterone and testosterone bound to endogenous binders. The Examiner also will appreciate from reading pages 25-27, that if the labeled testosterone analog is able to bind with endogenous binder, a false measurement of free testosterone will result. However, when sulfobromophthalein is present as a blocking agent, the blocking agent inhibits the binding of the radiolabeled testosterone analog (analog tracer) to the endogenous binder (primarily, albumin) without displacing testosterone naturally bound to the endogenous binder. This method as recited in the claim closely tracks the disclosure at pages 25-27. Specifically, the last or "improvement" clause is directly based on Specification at p. 26, Table 21 which recites:

> ". . . since sulfobromophthalein inhibits the binding of the analog tracer to albumin without displacing testosterone bound to albumin."

It is sincerely submitted that the claim is well grounded in the Specification and there is no new matter.

Turning to Paragraph 9 of the Office Action

(a) "derivative of testosterone", now "analog" of testosterone, is further defined in the claim as being "radioiodinated 6-hydroxy-testosterone-19 carboxymethyl ether histamine", lines 5-6. There is no uncertainty about what the analog is.

(b) The claim has been revised to uniformly recite "free portion of testosterone";

(c) The term "substantially" no longer appears in the claims.

Turning to paragraph 10, the typos noted have been corrected.

The Notice of Allowance is requested.

Respectfully submitted,

Date: March 4, 2002

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MARKED UP VERSION OF AMENDMENTS TO CLAIM SET

54. (Amended) A method of determining the concentration of [a]the free portion of testosterone in a biological fluid, wherein said free portion of testosterone is in equilibrium with another portion of the testosterone bound to one or more endogenous binders in said fluid comprising the steps of (a) forming a mixture of a sample of said fluid with (1) an amount of a specific antibody for the free portion of testosterone insufficient to substantially affect said equilibrium, and (2) a labeled [derivative]analog of testosterone which is radioiodinated 6-hydroxy-testosterone-19-carboxymethyl ether histamine that binds to said antibody and has affinity for the engodenous binders lower than that of the free portion of testosterone for said endogenous binders, (b) [maintained]maintaining said mixture to permit said labeled [derivative]analog to [complete]compete with the free portion of testosterone for binding with the antibody, (c) measuring the amount of said labeled [derivative]analog that has, or has not, become bound to the antibody, and (d) determining the concentration of the free portion of testosterone from said measurement, wherein the improvement comprises including in the mixture an amount of a blocking agent which is sulfobromophthalein [which substantially reduces]to inhibit the binding of said labeled [derivative]analog to the endogenous binders without [substantially reducing the binding of]displacing testosterone bound to said endogenous binders.